THE CONVERSION OF CORN STOVER AND PIG MANURE TO CARBOXYLIC ACIDS WITH THE MIXALCO PROCESS

A Senior Honors Thesis

By

AMANDA SPRING BLACK

Submitted to the Office of Honors Programs
& Academic Scholarships
Texas A&M University
In partial fulfillment of the requirements of the

UNIVERSITY UNDERGRADUATE
RESEARCH FELLOWS

April 2000

Group: Engineering

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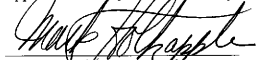
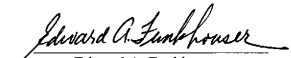
Approved as to style and content by:



Mark T. Holtzapple
(Fellows Advisor)



Edward A. Funkhouser
(Executive Advisor)

April 2000

Group: Engineering

ABSTRACT

The Conversion of Corn Stover and Pig Manure to

Carboxylic Acids with the MixAlco Process. (April 2000)

Amanda Spring Black
Department of Chemical Engineering
Texas A&M University

Fellows Advisor: Dr. Mark T. Holtzapple
Department of Chemical Engineering

The MixAlco process, developed by Dr. Mark T. Holtzapple, uses anaerobic fermentation to convert waste biomass into carboxylate salts which can then be manipulated into carboxylic acids, ketones and alcohols. This project focuses on the application of these processes to a feedstock of corn stover and pig manure.

During fermentation, corn stover was the energy source (carbohydrates) and pig manure was the nutrient source (vitamins, minerals, and growth factors). A countercurrent fermentation procedure was employed, using a four-reactor system, to prevent to inhibitory effects of high product concentrations. Lime pretreatment of both the corn stover and the pig manure aided in digestibility.

Batch tests showed that a substrate concentration of 40% corn stover to 60% pig manure in the system produced the highest conversion and yield. Subsequent testing revealed that the addition of nutrients and urea to the system also resulted in higher conversion, although the reduction in product concentration when omitting the nutrients was minimal.

The highest average acid concentration produced by a countercurrent fermentation of 40% corn stover/60% pig manure was 28 g carboxylic acid/L liquid. This steady state acid concentration was reproduces during two separate periods of steady state. Conversions as high as 68% were achieved.

It was hypothesized that sonicating biomass during the fermentation procedure could act as a cleansing mechanism—removing components from the surface of the biomass that inhibit further digestion. Initial testing showed no increase in product concentration or conversion; however, an increase in yield was noted.

ACKNOWLEDGMENTS

I would especially like to thank Susan Burdick, now Dr. Susan B. Domke, for helping me begin my research in 1998, and for continuing to be a patient and helpful mentor for the next year and a half.

I would also like to thank the graduate students who have helped me while working in the laboratory: Cateryna Aiello, Sally Chan, and Piyarat Thanakoses. Thank you for your assistance and flexibility. I also appreciate all of the student workers who helped me maintain my trains, especially during the last few months.

Finally, I would very much like to thank my advisor, Dr. Mark T. Holtzapple for first convincing me to remain in chemical engineering and then introducing me to his fascinating research. Thank you for allowing me to begin researching when others thought I was too young, and enabling me to experience a rewarding two years.

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CHAPTER I

INTRODUCTION

In an age of rapid technological development, the human population is plagued by several issues resulting from the pursuit of an easier way of life. One issue—pollution—has become very serious over the past century. In 1980, the United States alone produced approximately 558 million dry tons of waste biomass per year (Cheremisinoff, 1980). Approximately 30% of this waste was municipal solid waste (MSW), or "trash." The remainder consists of agricultural and forestry residues, sewage, and manure (Cheremisinoff, 1980).

These products are termed "wastes" because they have a negative value to society due to costs incurred through their disposal. Traditional means of disposal include land filling or incineration, both of which can negatively affect the environment and quality of life. The amount of waste produced annually is increasing, and current disposal methods are sufficient, but non-ideal.

Since the early 1900s, scientists have searched for a method to use waste materials as a resource. For the past decade, Dr. Mark T. Holtzapple of Texas A&M and his colleagues have been implementing a series of patented technologies labeled the MixAlco process that can convert negative-value biomass into useful resources (Holtzapple, 1998).

This thesis follows the style of *Biotechnology and Bioengineering*.

In the MixAlco process, the biomass is lime treated to aid digestibility. The lime-treated agricultural residue may be employed as ruminant animal feed. Alternately, the treated biomass can be fermented using a culture of ruminant bacteria to digest the biomass into carboxylate salts (e.g., calcium acetate, propionate, and butyrate). These carboxylate salts can be chemically manipulated to produce ketones or alcohols, both of which have a marketable value.

Benefits of the process include the positive value of its products, as well as positive effects on the economic and environmental aspects of waste disposal. The resulting ruminant animal feed lessens the need for agricultural crops grown solely as animal feed. Further, the chemicals and fuels may be put to a variety of uses.

THE MIXALCO PROCESS

The MixAlco process consists of several related steps. Biomass progresses through a series of reactions, each of which results in a product that either advances to the next process step, or is collected as a useful resource (Figure I-1).

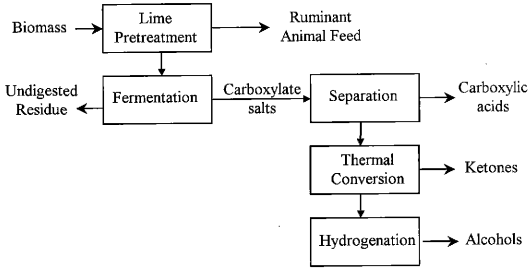

Figure I-1: The MixAlco process.

Biomass

As previously mentioned, biomass exists in several forms: municipal sewage sludge, industrial biosludge, paper, manure, agricultural residue, and organic MSW such as food waste, packaging, and lawn clippings. For the MixAlco process, biomass is classified as an energy source or a nutrient source.

Energy sources are high in carbohydrates needed to provide the energy to sustain a culture of fermentation microorganisms. Typical energy sources include agricultural residues, paper and packaging. In contrast, nutrient sources are low in carbohydrates, but high in nutrients (e.g., vitamins, growth factors, minerals, and nitrogen). Manure, biosludge, and sewage sludge constitute good nutrient sources.

A single source, either energy or nutrient, is not able to sustain a culture at optimal digestion levels. Past research suggests that combining both an energy and nutrient source at specific ratios allows for a more complete digestion of the total biomass during fermentation (Rapier, 1995). Therefore, it is necessary to specify an optimal combination of biomass types.

Lime Treatment

Much of the candidate biomass is lignocellulose, a material consisting of cellulose, lignin, and hemicellulose (Holtzapple, 1997). Lignocellulose has a characteristically low digestibility due to digestion-inhibiting acetates on hemicellulose, and lignin which is indigestible.

In the lime treatment step of the MixAlco process, the biomass is alkali treated to increase its enzymatic digestibility (Holtzapple, 1997). Lime is used due to its low cost and process compatibility. Also, residual lime is later neutralized by acids produced during the fermentation procedure, allowing for efficient product recovery.

Lime treatment removes lignin and acetate from the hemicellulose, resulting in biomass with a greater reactivity than the original untreated biomass. Past research shows that lime pretreatment approximately doubles the digestibility of some agricultural residues and increases MSW digestibility by 1.1 to 1.3 times (Gandi, 1997). Although lime pretreatment does significantly increase the digestibility of the biomass, approximately 20% of most biomass is composed of lignin—an indigestible substance

(Saha, 1997). For this reason, no biomass is 100% digestible. There is always a lignin and ash residue.

Certain lime-treated agricultural residues may be removed from the process at this point and used as ruminant animal feed. Alternately, the pretreated biomass can proceed to the fermentation step.

Fermentation

The fermentation uses a mixed culture of anaerobic acid-forming microorganisms, much like those in the digestive tract of ruminant animals. Ruminant bacteria are inexpensive and easily obtained. The mixed culture is beneficial because it adapts to a wide variety of input materials while regenerating its population to maintain an equilibrium.

The microbial digestion of biomass results in carboxylic acids (e.g., acetic, propionic, and butyric acids). However, as digestion progresses and more acids are produced, the pH reduces and threatens to inhibit further digestion. To maintain optimum acid production, the fermentation is conducted in a train of four countercurrent fermentors. Solids traverse across the train in a direction opposite to that of the liquids (Figure I-2).

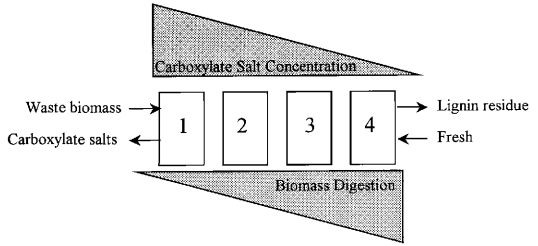

Figure I-2: Countercurrent effects on acid concentration (Domke, 1999).

The countercurrent fermentation system effectively allows the production of high-concentration carboxylate salts because it places the water with the lowest product concentration in Fermentor 4, which contains the most fully digested solids, as depicted in Figure I-2. This lessens the product inhibition that would be present in Fermentor 4 if the liquid already contained high product concentrations. In Fermentor 1, although the product concentration is high, the biomass is very digestible, allowing for finite reaction rates.

To maintain an appropriate pH for microorganism growth, the carboxylic acids produced during digestion are neutralized to carboxylate salts. These carboxylate salts exit with the liquid leaving Fermentor 1 and proceed to the next step in the process.

Carboxylate Salt Conversion

The carboxylate salts exiting the fermentation train are dewatered and concentrated. They may be converted to acetic, propionic, butyric, and other higher acids. Alternately, the carboxylate salts may be thermally converted to form ketones, such as acetone, methyl ethyl ketone, and diethyl ketone. Subsequent hydrogenation of the ketones results in the corresponding alcohols isopropanol, isobutanol, and isopentanol. The resulting chemicals and fuels can then be sold for a profit.

The MixAlco process, though proven in the laboratory, has not yet been implemented on a larger scale. As experimentation proceeds to the pilot plant level, the goal is to improve process efficiency. One way to create a more efficient process is to maximize the degree of digestion during the fermentation. High product output is also desirable. It is necessary to perform the process using a feedstock combination that is readily available and produces high product concentrations.

This project examines the fermentation, investigates the viability of a corn stover and pig manure feedstock combination, and tests the effects of including a sonication procedure during fermentation.

CHAPTER II

MATERIALS AND METHODS

This chapter first discusses the selection, collection, and treatment of biomass used during the project. Then, the many procedures necessary to conduct a fermentation are described. Procedures for analyzing the liquid, solid and gas output from the fermentors are also explained. Finally, methods for conducting a mass balance on the reactors are clarified.

SELECTION, COLLECTION, AND TREATMENT OF BIOMASS

As discussed previously, in the MixAlco process, the biomass feedstock must contain an energy and nutrient source. Most agricultural residues are energy sources, whereas manure is an optimal nutrient source. For this reason, agricultural residues and manures make a beneficial combination. Their availability is also complimentary, because most agricultural areas contain both crops and livestock.

Agriculture generates a large portion of the waste biomass produced annually. Of the crops grown in the United States, the number of bushels of corn produced annually is more than double that of any other grain crop. In 1999, the United States produced 240 million tons of corn (United States Department of Agriculture, 2000). Of this production, over 50% originated from the Corn Belt states, particularly Illinois, Indiana, Iowa, Minnesota and Nebraska. Table II-1 depicts the corn production for these states in 1999.

Table II-1: Corn acreage and production in the United States in 1999 (United States Department of Agriculture, 2000).

	Acres of Corn Crops (1000 acres)	Percent of National Total (%)	Bushels Produced (100000 bushels)	Percent of National Total (%)
Illinois	10800	14.0	1491.0	15.8
Indiana	5600	7.2	748.4	7.9
Iowa	12100	15.6	1758.2	18.6
Minnesota	7100	9.2	990.0	10.5
Nebraska	8600	11.1	1157.7	12.3
TOTAL	44200	57.1	6145.3	65.1

Corn production is very concentrated in these states, making corn stover—the residue remaining after corn is harvested—a possible energy source for the MixAlco process.

Table II-2 depicts hog production for the same five states. These same five states produced over 50% of the swine sold in the United States in 1998. The massive swine production present in these states generates large amounts of manure that may be used as a nutrient source in the MixAlco process.

Table II-2: Hog farms and production in the United States in 1998 (United States Department of Agriculture, 2000).

	Hog Raising Farms (Number)	Percent of National Total (%)	Hogs Sold (1000 Head)	Percent of National Total (%)
Illinois	7000	6.1	4850	7.8
Indiana	6400	5.6	4050	6.5
Iowa	17500	15.3	15300	24.6
Minnesota	6000	5.2	3400	5.5
Nebraska	8500	7.4	5700	9.2
TOTAL	45400	39.7	33300	53.5

Corn stover and pig manure are both concentrated in the midwestern states, and therefore are a readily available source of waste biomass that may be disposed of through the MixAlco process. This project examines their suitability for the fermentation process.

Corn stover was obtained through the Ames Research Laboratory at Iowa State University. It was shipped by mail, and received dry and whole, and was then ground to a fine particle size. The ground corn stover was then treated with lime to increase digestibility. (A complete description of the lime pretreatment process is located in Appendix A.) The treated corn stover was then dried in a 105 °C oven for two days and tested for moisture content. The final corn stover entering the process had an average of 0.093 g water/g corn stover and 0.119 g ash/g corn stover.

The pig manure was collected at the Texas A&M University Swine Center (contact: Kenton Lillie, 979-842-4736). The fresh manure was allowed to air dry for a

period of three to six days, after which it was treated with the lime pretreatment procedure described in Appendix A. The treated manure was then dried in a 105 °C oven for two days and broken by hand into small pieces. The moisture content was an average of 0.016 g water/g pig manure and the ash content was 0.221 g ash/g pig manure. Table II-3 depicts the dry and ash weights of both feedstock.

Table II-3: Composition of feedstock.

Fedstock	Dry Weight (g/g treated biomass)	Ash Weight (g/g treated biomass)
Corn Stover	0.907	0.119
Pig Manure	0.984	0.221

FERMENTATION

The fermentation was conducted in a "train" consisting of four individual fermentors, following the design specifications of Ross (1998) and Domke (1999). Each fermentor was constructed from a 1-L polyethylene terepthalate centrifuge bottle equipped with a metal bar for stirring. The bottle was sealed with a Size 11 rubber stopper to prevent gas leakage, and the stopper was secured by the original bottle lid which was modified with a 5-cm hole in the center. This fermentor is impermeable to oxygen and has a septum for sampling and venting excess gas. The rubber septum was connected to the fermentor by a glass tube inserted through a hole in the rubber stopper.

Each fermentor was stored in an incubator at 40 °C and rotated at 1 rpm on a Mode III Wheaton Modular Cell Production Roller Apparatus (Domke, 1999). Figure II-1 illustrates the individual parts of the total fermentor. The procedure for constructing fermentors is located in Appendix B.

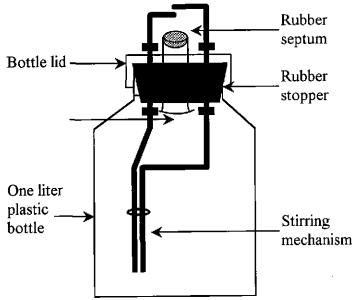

Figure II-1: Components of the fermentor design utilized.

Fermentor Inoculation

Each fermentor was initiated with a specified amount of biomass at the desired ratio of corn stover to pig manure. Subsequent chapters discuss the different weights and ratios studied. The biomass was added to a media of deoxygenated, distilled water, with sodium sulfide and cysteine hydrochloride. This media traps any oxygen molecules that enter the system, and provides the appropriate anaerobic environment for

the bacteria that were introduced with the inoculum. Media preparation techniques are discussed in Appendix C.

The inoculum used in this project was collected from a several sources to obtain the widest variety of organisms for the mixed culture. The primary source of inoculum was rumen fluid from a fistulated steer located at the University Nutrition and Field Laboratory at Texas A&M University (College Station, TX). The rumen fluid was collected by hand and stored in bottles for transport back to the laboratory, where it was immediately added to the fermentors.

Other sources of inoculum included swamp matter from Wolf Pen Creek, College Station, Texas, humus from Dr. Mark Holtzapple's garden, and compost from Dr. Holtzapple's residence. These substances were collected in bottles containing deoxygenated, distilled water with sodium sulfide, and cysteine hydrochloride (Appendix C)—maintaining an anaerobic environment for the organisms in the inoculum, and preserving them during transport back to the laboratory for addition to the fermentors.

Supplemental Additions to the Fermentors

Calcium carbonate was added to the fermentors both at their initiation and periodically throughout the fermentation. The calcium carbonate converted some of the carboxylic acids produced by the microorganisms during digestion to carboxylate salts, thereby neutralizing the acid. This was necessary to maintain the pH at an appropriate level of 5.5 or above, and avoid the inhibiting effects of low pH.

Urea was also added to each individual fermentor periodically over the lifespan of the system to provide nitrogen for the bacteria. However, excess urea increased the pH, which could be hazardous to the microorganisms. For this reason, pH was closely monitored and urea was not added if the pH exceeded 6.9.

Past research by Ross (1998) has revealed that supplementary nutrients, in addition to those contained in the nutrient biomass source, create higher acid output concentrations. Specifically, Ross recommends a dry, modified Caldwell and Bryant medium. The components and preparation instructions for the modified Caldwell and Bryant medium are listed in Appendix C. Nutrients, in this form, were added to all fermentors, both at initiation and periodically over their lifespan.

Iodoform, in the form of a 20 mL iodoform/L ethanol solution, was added to each of the fermentors periodically depending on the specifications of the train. The iodoform acts as a methane inhibitor. Methane is an undesirable product because it utilizes the carbon atoms that otherwise would be present in the form of a carboxylic acid.

Batch Fermentation

During batch fermentation, no mass entered or left the system, with the exception of the necessary supplemental additions (nutrients or iodoform) and small amounts of liquid removed to test the acid concentration. The gas was also vented and measured occasionally to prevent the container from bursting.

Each new fermentation train began with the four individual fermentors running in a batch mode for a period of two weeks. This enabled the mixed bacteria culture to grow and strengthen while adapting to the feedstock of corn stover and pig manure. After a culture was established, the system was run in the countercurrent method.

Other small batch experiments were performed over a two-week period to test several corn stover to pig manure ratios, as well as to confirm that adding urea and nutrients were beneficial. The results are discussed in the subsequent chapter.

Countercurrent Method

Each fermentation train existed in batch mode for the initial two weeks, after which solid and liquid transfer began in a countercurrent fashion as depicted previously in Figure 1-2. The fermentors were operated with an equal and constant mass of solid and liquid. Every other day, solids and liquids flow in the countercurrent method with the double-centrifuge procedure described in detail in Appendix D.

As depicted in Figure I-2, the solids move from left to right. Fresh biomass is added to Fermentor 1, and in order to keep mass constant, some solids in Fermentor 1 are removed and transferred to Fermentor 2, Fermentor 2 to Fermentor 3, and Fermentor 3 to Fermentor 4. Solid residue is removed from Fermentor 4 and retained for analysis. Therefore, the freshest biomass is contained in Fermentor 1, and the most digested solids are found in Fermentor 4.

Conversely, the liquids move from right to left. Liquid containing the product (carboxylate salts) is decanted from Fermentor 1 and progresses to the next step in the

MixAlco process. Liquid from Fermentor 2 is then added to Fermentor 1, Fermentor 3 to Fermentor 2, and Fermentor 4 to Fermentor 3. Finally, fresh media is added to Fermentor 4. Therefore, the liquid with the highest concentrations of carboxylate salts is found in Fermentor 1 because that liquid has been in the system the longest.

The concentration of acids received and the amount of residue remaining depends on the residence time of the solids and liquid—the time they remain in the fermentor train. A typical liquid residence time is 12 to 20 days, whereas the typical solid residence time is 1 to 2 months. As solid residence time increases, the concentration of liquid products will drop because the solids will have been in the system longer and will have become more digested. If solid residence time is shortened, then the product concentrations will be higher, but less of the solids digest, thus increasing the amount of residue.

Gas Measurement

The gas must be vented from the fermentors every time they are opened for the transfer process. To measure the amount of gas produced, a needle connected to a gas measurement apparatus is inserted into the rubber septum of a fermentor which had cooled to room temperature. The gas measurement apparatus consists of a glass cylinder connected to both a vacuum and a water supply (Figure II-2).

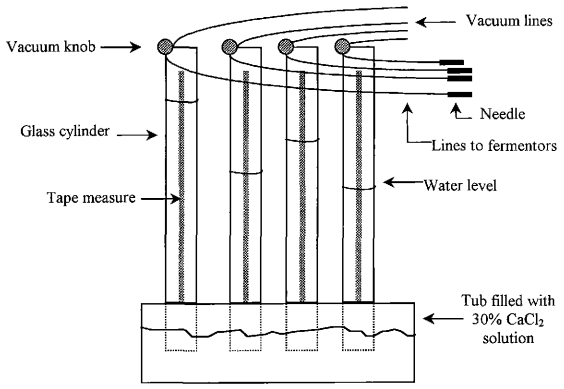

Figure II-2: Gas measurement apparatus.

The vacuum is used to fill the cylinder with water. Then the fermentor gas displaces the water, and the gas height is measured in cm. The inside diameter of the glass tube is 50 mm, and therefore each cm displaced represents 19.6 mL of gas (Ross, 1998). However, inserting the rubber stopper into the top of the fermentation bottle causes some increase in pressure, which could effect the gas measurements. Testing depicted that placing the stopper into the bottle caused an average of 3 cm pressure increase for Fermentors 1, 2 and 3, and a 2 cm pressure increase for Fermentor 4. These numbers were subtracted from the measurements before the gas volume was calculated.

Some gas leakage did occur, because occasionally gas measurements were less than 3 cm. Subtracting from this value would result in a negative gas production, which is not possible. In this circumstance, the gas production was assumed to be zero. The error associated with this assumption should be small, and not affect the overall mass balance.

ANALYTICAL TECHNIQUES

All of the liquid, gas, and solid residue from the fermentation system was collected and analyzed. The liquid was tested to determine the carboxylic acid concentrations. Both liquid and solids were tested to determine the amount of volatile solids (VS) that exited the system. Volatile solids include the digestible portion of the biomass, plus the lignin. The gas was also analyzed to determine the content.

Liquid Analysis, Carboxylic Acid Concentration

Every transfer period, a small sample of liquid from Fermentor 1 was collected in a test tube to measure the carboxylic acid output of the train. Gas chromatography was used to test the sample for the presence of acetic, propionic, butyric, valeric, caproic, and heptanoic acids. The gas chromatograph was a Hewlett Packard 5890A utilizing a flame ionization detector and a Hewlett Packard 7673A autosampler (Domke, 1999). The column pressure was 90-103 kPa.

VS Analysis

The remaining amount of liquid exiting Fermentor 1 and the solid residue exiting Fermentor 4 were stored in collection bottles. The liquid contained both dissolved VS and particulate VS that were decanted with the liquid during the transfer process. Due to the presence of carboxylic acids, the liquid must first be treated with lime to prevent the acids from volatizing (Ross, 1998). The solid residue is analyzed without the presence of lime.

VS analysis was conducted by drying the matter in an oven at 105 °C for at least 48 hours and then ashing at 550 °C for at least three hours. The volatile solids were determined by the dry weight minus the ash weight (Ross, 1998). Thorough descriptions of the procedures are contained in Appendix E.

Gas Composition Analysis

Periodically, gas chromatography is used to analyze the composition of the off-gas generated by the fermentors. The three major components of the off-gas were methane (CH_4), carbon dioxide (CO_2), and nitrogen (N_2). Methane and carbon dioxide are both products of microbial digestion. The nitrogen was present due the constant nitrogen purge used when the fermentors were opened to the atmosphere. Knowledge of the composition and amount of gas exiting the fermentor enabled calculation of the amounts of both CO_2 and CH_4 leaving the system.

MASS BALANCE TECHNIQUES

To determine the amount of digestion occurring in the fermentation system, a mass balance was conducted on the entire train over a steady-state period. The mass balance determined the difference between the mass entering the system and the mass exiting the system. Initially, biomass enters the system as volatile solids and ash. During digestion, the biomass is converted to several different products as depicted in Figure II-3.

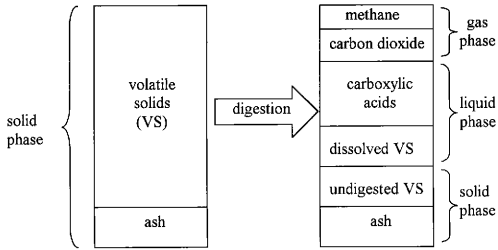

Figure II-3: Products of fermentation (Domke, 1999).

The mass of each of the products exiting the system was determined with the values recorded during the transfer and analysis procedures. These values were used to calculate the percent closure of the system.

Closure helps determing the accuracy of the measurements, and is defined as

$$\text{closure} \equiv \frac{(\text{undigested VS} + \text{dissolved VS} + \text{acids} + \text{biotic } CO_2 + CH_4)}{(\text{VS in} + \text{water of hydrolysis})}$$

The system must obey the law of conservation of mass, and should theoretically have 100% closure. Any discrepancies in the closure value are due to error in the measurements.

When calculating closure, it was necessary to distinguish between biotic and abiotic CO_2. Biotic CO_2 is actually produced by the microorganisms during fermentation—and is therefore a product of the process. Abiotic CO_2 is a result of the neutralization of acids by the calcium carbonate added to the system, and cannot be considered a product. This reaction is governed by the following equation (Ross, 1998):

$$2CH_3(CH_2)_xCOOH + CaCO_3 \rightarrow Ca(CH_3(CH_2)_xCOO)_2 + H_2O + CO_2$$

The stoichiometry suggests that one mole of abiotic CO_2 is produced for every 2 moles of acid (Ross, 1998). Ross (1998) also offers an approximation for the water of hydrolysis

$$\text{water of hydrolysis} = \text{VS digested} \times \frac{18}{162}$$

which accounts for the mass increase when carbohydrate polymers (cellulose and hemicellulose) are hydrolyzed to sugars.

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Other terms utilized in analyzing the data are defined as follows:

$$\text{conversion} \equiv \frac{\text{VS digested}}{\text{VS fed}}$$

$$\text{yield} \equiv \frac{\text{total carboxylic acids produced}}{\text{VS fed}}$$

$$\text{selectivity} \equiv \frac{\text{total carboxylic acids produced}}{\text{VS digested}}$$

CHAPTER III

BATCH EXPERIMENTS

Two different batch studies were conducted during to determine optimal operating parameters for a corn stover/pig manure system. The first test studied the ratio of corn stover to pig manure that should be contained in the system. The second test investigated the effects of nutrients and urea in different amounts and combinations to determine if their addition was beneficial.

CORN STOVER TO PIG MANURE RATIO

Rapier suggested 80% MSW (energy source) and 20% SS (nutrient source) as the ideal combination of energy and nutrients (1995). Theoretically, the ratio of energy to nutrient source that produces the best results will vary with the individual feedstock. Five batch reactors were used to test the following ratios of corn stover/pig manure: 80/20, 60/40, 40/60, and 20/40. The fifth reactor was operated with an 80/20 ratio; however, the pig manure had not been treated with lime before entering the fermentation process. This batch reactor was used to determine if lime treating the manure was necessary.

Each individual reactor was initiated with 30 g of substrate in the appropriate ratios, 250 mL of distilled deoxygenated water with sodium sulfide and cysteine hydrochloride, and 50 mL of inoculum from an existing fermentation train of 80% corn stover/20% pig manure. This corresponded to an initial substrate concentration of

100g/L. The batch reactors were also supplemented with 2.0 g calcium carbonate, 0.2 g urea, 0.2 g dry Caldwell and Bryan nutrient mix, and 40 μL of 20 mL iodoform/L ethanol solution. The supplements were added at the initiation of the reactors, and every other day during the two-week testing period.

Figures III-1 to III-5 display the acid concentrations of the five fermentors, and the mass balance is summarized in Table III-1. The results suggest that a higher concentration of nutrient source is necessary for maximum product concentration in a corn stover and pig manure fermentation. The ratio of 40% corn stover/60% pig manure performed the best, producing the highest overall acid concentration, and yield.

The conversion percentages were similar between the fermentors at 80/20, 60/40, and 40/60 corn stover/pig manure ratios—all converting at close to 50%. The only notably low conversion rate occurred in the fermentor containing the untreated pig manure, suggesting that lime treatment does increase digestibility and is necessary if a high conversion is desired.

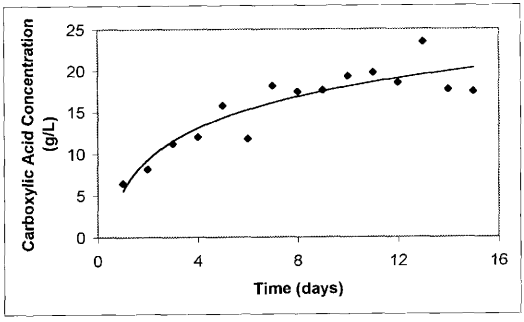

Figure III-1: 80% corn stover/20% pig manure batch fermentor.

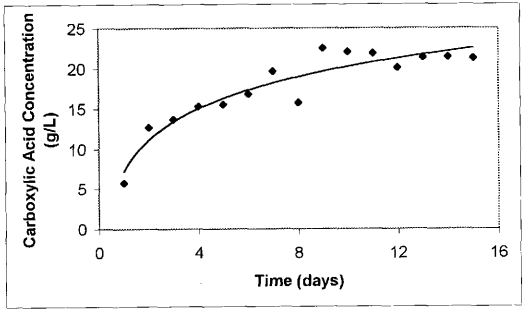

Figure III-2: 60% corn stover/40% pig manure batch fermentor.

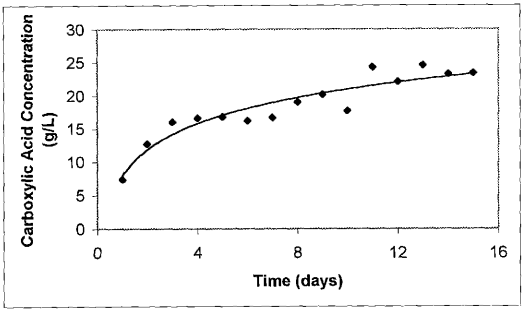

Figure III-3: 40% corn stover/60% pig manure batch fermentor.

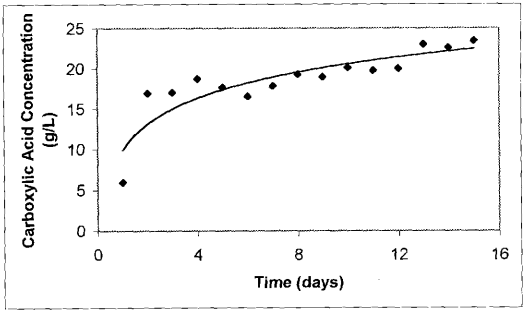

Figure III-4: 20% corn stover/80% pig manure batch fermentor.

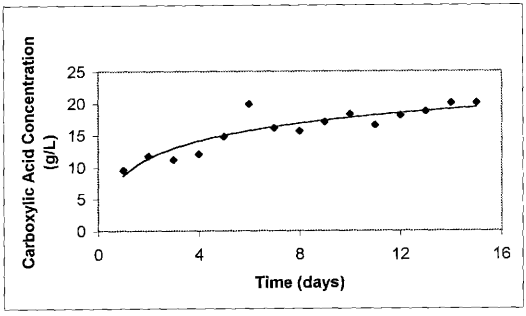

Figure III-5: 80% corn stover/20% untreated pig manure batch fermentor.

Table III-1: Corn stover/pig manure ratio comparison.

Ratio, (% Corn Stover/ % Pig Manure)	Initial Substrate (g/L)	Final Total Acids (g/L)	Conversion (g VS digested/ g VS fed)	Total Acid Yield (g total acids/ VS fed)	Total Acid Selectivity (g acids/ VS digested)
80/20	100	20.0	45.8	13.0	28.3
60/40	100	22.5	50.8	14.8	29.1
40/60	100	24.0	50.0	16.1	32.3
20/80	100	23.0	52.9	15.3	28.9
80/U20	100	20.0	38.7	12.2	31.6

NUTRIENTS AND UREA REQUIREMENTS

Past research conducted by Ross (1998) and Domke (1999) reports a need for both nutrient and urea supplementation during fermentation. Seven batch reactors were used to determine if nutrients (the Caldwell and Bryant medium) and urea were necessary in a corn stover and pig manure fermentation, and in what amounts. Each individual reactor was initiated with 30 g of substrate in a 40% corn stover/60% pig manure ratio, 10 mL of rumen fluid, 10 mL of inoculum from an existing 40% corn stover/60% pig manure fermentation, and 280 mL of distilled, deoxygenated water with sodium sulfide and cysteine hydrochloride. This corresponded to an initial substrate concentration of 100 g/L. Supplementation included 2.0 g calcium carbonate and 40 µL of 20 mL iodoform/L ethanol solution added at the initiation of the reactors and every other day over the two-week testing period. Nutrients and urea were also added (in the

amounts depicted in Table III-2) at initiation and three other times over the subsequent two weeks.

Table III-2: Nutrient and urea additions to batch fermentors.

Fermentor Lable	**Nutrient Addition (g)**	**Urea Addition (g)**
N	0.2	---
U	---	0.2
N-U	0.2	0.2
2N	0.4	---
2N-U	0.4	0.2
3N	0.6	---
3N-U	0.6	0.2

Nutrient Requirements

Figure III-6 compares Fermentor U, which received no nutrients, to Fermentor NU, which received 0.2 g nutrients. Although Fermentor U obtained a higher carboxylic acid concentration in a shorter period of time, Fermentor NU surpassed U in acid production after the first week, and the fermentor receiving the nutrients resulted in a slightly higher trend in carboxylic acid production.

Results from the mass balances of all seven fermentors are displayed in Table III-3. These results show that the fermentation supplemented with nutrients had a conversion 5% greater than the conversion of the fermentation receiving no nutrients. The yield was also greater, but only by 1%.

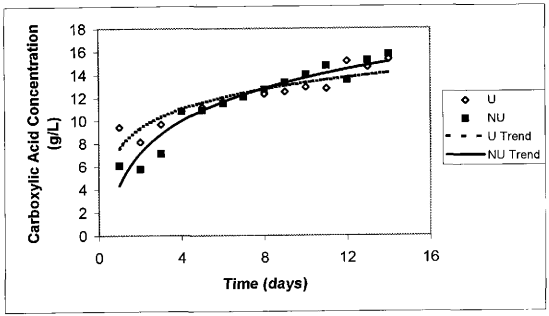

Figure III-6: Determining the necessity of nutrients.

Table III-3: Nutrients and urea addition comparison.

Ratio, (% Corn Stover/ % Pig Manure)	Initial Substrate (g/L)	Final Total Acids (g/L)	Conversion (g VS digested/ g VS fed)	Total Acid Yield (g total acids/ VS fed)	Total Acid Selectivity (g acids/ VS digested)
N	100	14.7	45.8	8.8	19.1
U	100	15.3	46.8	9.4	20.1
N-U	100	15.6	51.4	10.3	20.0
2N	100	15.5	44.3	9.6	21.6
2N-U	100	16.9	44.0	10.3	23.4
3N	100	15.3	40.5	8.6	21.3
3N-U	100	14.5	44.0	9.0	20.5

Figure III-7 compares the carboxylic acid concentrations of a group of three fermentors (N, 2N, and 3N) that received no urea and different amounts of nutrients (as described in Table III-1). No one fermentor performed distinctly better than the rest. Fermentors N and 3N obtained a final acid concentration of 15.3 g/L while Fermentor 2N obtained only a slightly higher value—15.5 g/L. Fermentor N did have a higher conversion rate than Fermentors 2N and 3N, but only by 2%.

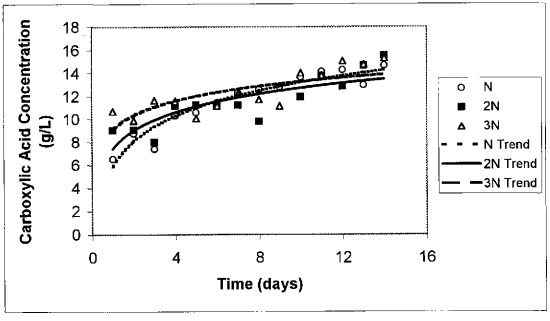

Figure III-7: Comparing the effects of higher nutrient addition.

Figure III-8 compares the carboxylic acid concentrations of a group of three fermentors (N-U, 2N-U, and 3N-U) that received 0.2 g urea and varying amounts of nutrients (as described in Table III-1). Fermentor 3NU performed poorly when compared to Fermentors NU and 2NU, producing a lower carboxylic acid production trend. Fermentors NU and 2NU performed similarly, obtaining the highest final acid concentrations of all seven batch reactors. Fermentor 2N had a slightly higher final product concentration, and Fermentor N obtained a higher conversion, but only by 2%.

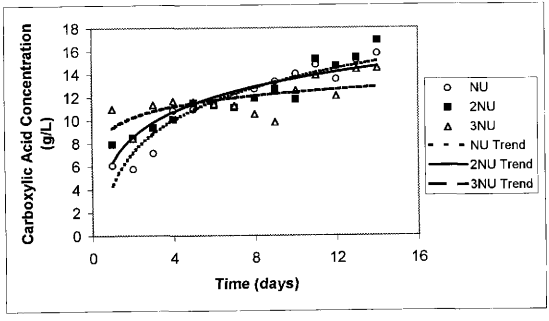

Figure III-8: Comparing the effects of higher nutrient addition in the presence of urea.

With and without the addition of nutrients, Fermentor 3N performed the worst. However, it is difficult to distinguish between the Fermentors N and 2N. Fermentor N seems to offer a higher conversion rate, whereas Fermentor 2N offers greater acid production.

Urea Requirements

Optimal performance occurred in the fermentors recieving 0.2 or 0.4 g of nutrients every other day during the testing period. Figures III-9 and III-10 compare two fermentors at each of these values. Of the two fermentors, one received only the nutrients supplement every other day and no urea, while the other received 0.2 g urea every other day as well as the nutrients supplement.

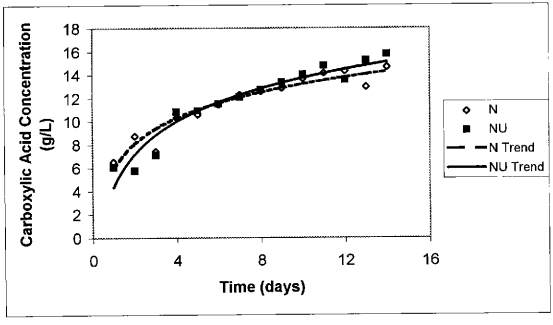

Figure III-9: Analyzing the effects of urea with a nutrients supplement of 0.2 g/2 days.

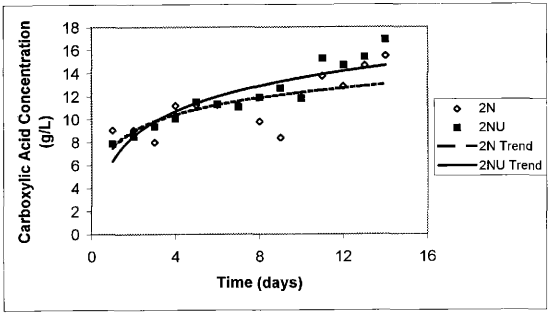

Figure III-10: Analyzing the effects of urea with a nutrients supplement of 0.4 g/2 days.

In both cases, the fermentor receiving urea performed better than the fermentor receiving no urea. The final carboxylic acid production of Fermentor NU was 0.9 g/L higher than that for Fermentor N, and Fermentor 2NU was 1.4 g/L higher than Fermentor 2N. Conversion and yield were also increased by adding urea.

CHAPTER IV

SONICATION EXPERIMENT

The biomass exiting Fermentor 3 is highly digested; however, some portions of the solids may be blocked from further digestion. During digestion, microorganisms attach themselves onto the surface of the digestible biomass. They break-down the biomass producing sugars, which they absorb for nutrients. To prevent other microorganisms from absorbing its sugars, a single organism will form a protective shell around an area to trap the sugars it produces, as shown in Figure IV-1.

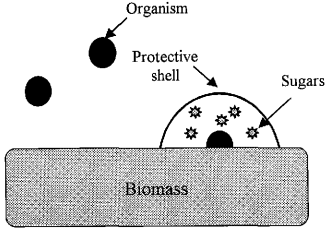

Figure IV-1: Bacterial digestion of biomass.

During the fermentation of the MixAlco process, microorganisms are subjected to harsh conditions. The environment is acidic, at a pH between 5.5 and 6.5 with a high product concentration. Harsh conditions such as these may cause some microorganisms to die while the protective shell remains, preventing other microorganisms from reaching that area of biomass—essentially blocking off digestible regions of the biomass. If this is the case, removing the coating would result in a larger exposed area of digestible biomass.

Sonication may be a means of cleansing the biomass surface, removing any elements inhibiting digestion, such as the abandoned shells. Theoretically, bombarding the solid surface of the biomass with sonic waves will break off the protective shells and replenish the surface for further digestion, as depicted in Figures IV-2 and IV-3.

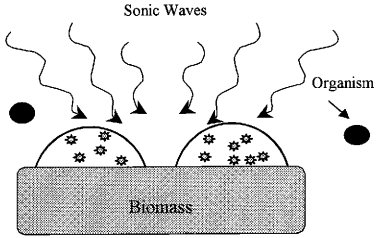

Figure IV-2: Sonication

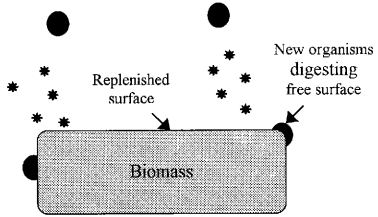

Figure IV-3: Free surface exposed after sonication.

SONICATION METHODS AND RESULTS

Two countercurrent fermentation trains were initiated to test the effects of sonication on the MixAlco process. Both trains were initiated with 50 g of feedstock from a previous fermentation train operated at 40% corn stover/60% pig manure ratio and 30 g of fresh feedstock in a 40% corn stover/60% pig manure ratio. After two weeks in batch operation to allow for culture establishment, the countercurrent double-centrifuge procedure began. Both trains underwent the mass transfer process every other day, during which 20 g of fresh biomass and 200 mL fresh media were added to the train.

Figures IV-4 and IV-5 display acid concentrations of the control train, Train A, and the sonication train, Train B before the sonication procedure was initiated. After an initial adjustment period, both trains came to a steady-state existence (SS-I) with an acid concentration of approximately 28 g/L. Production results calculated over the steady-state period are presented in Table IV-1.

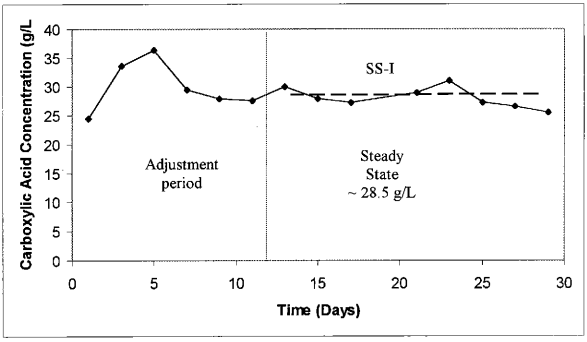

Figure IV-4: Carboxylic acid concentration for the control during SS-I.

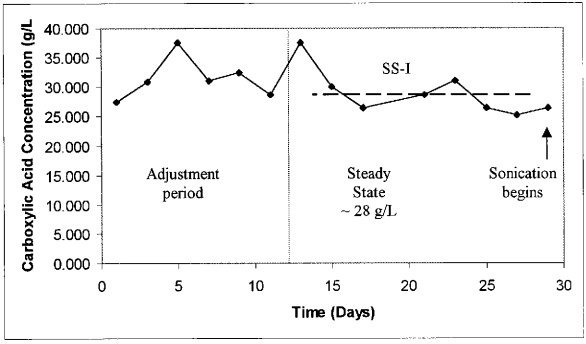

Figure IV-5: Carboxylic acid concentration for the sonicated train during SS-I.

Table IV-1: Results from SS-I, before sonication began.

Fermentor	Conversion (g VS digested/ g VS fed)	Yield (g total acids/ g VS fed)	Selectivity (g total acids/ g VS digested)
Control	49.8	30.6	61.6
Sonicated	68.1	30.3	44.5

At SS-I, before sonication began, both trains were running at favorable conditions.

Sonication was initiated in Train B on Day 30. Figures IV-6 and IV-7 depict the resulting acid concentrations of both Train A and Train B after Day 30. Initially, after sonication began, both fermentors showed a sharp increase in acid concentration—from 26 g/L to above 30 g/L. However, this was not due to the initiation of the sonication procedure, because only Train B received sonication; the trend was observed in both trains.

Between Days 50 and 80, the reactors were stored, an no mass transfer occurred until the process was reinitiated on Day 80. After an initial adjustment period, both trains reached a steady-state period (SS-II), again at an product concentration of approximately 28 g/L. Production results calculated over SS-II are presented in Table IV-2.

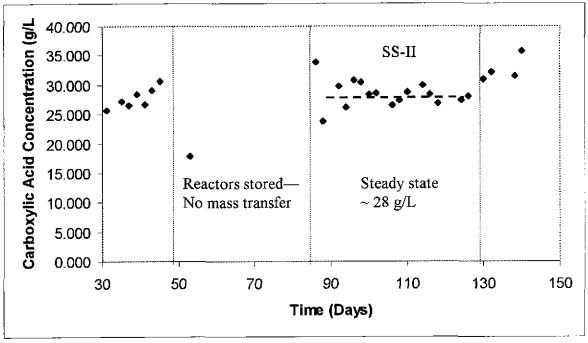

Figure IV-6: Carboxylic acid concentration for the control during SS-II.

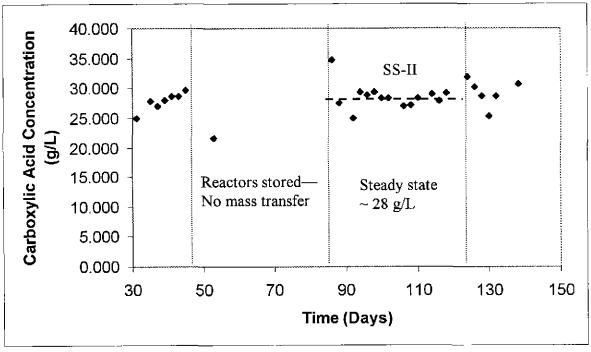

Figure IV-7: Carboxylic acid concentration for the sonicated train during SS-II.

Table IV-2: Results from SS-II, after sonication began.

Fermentor	**Conversion** (g VS digested/ g VS fed)	**Yield** (g total acids/ g VS fed)	**Selectivity** (g total acids/ g VS digested)
Control	48.7	19.4	40
Sonicated	39	22.7	57.1

Comparing SS-II to SS-I, the coversion decreased by 10% for Train A, and 29% for Train B. Yield and selectivity were also lower than SS-I. However, these trends were viewed in both trains, and therefore cannot be due to the sonication procedure.

CHAPTER V

CONCLUSIONS AND RECCOMENDATIONS

The following was learned from the batch studies:

1. Corn stover and pig manure are a favorable combination of energy and nutrient feedstocks.
2. A 40% corn stover/60% pig manure ratio is the most effective. However, in most states, the production of corn stover and pig manure corresponds to an approximate 80/20 ratio. Utilizing this ratio could result in a 5% reduction in conversion and a 1% reduction in yield. These differences can be considered insignificant if, by utilizing the available ratio, more biomass can be processed and hence, more product produced.
3. Lime treatment of the pig manure is necessary for the highest conversion percentage.
4. A fermentation of corn stover and pig manure requires the addition of both nutrients and urea for optimal performance.

The following was learned from the countercurrent studies:

1. A 40% corn stover/60% pig manure fermentation is capable of stabilizing at a carboxylic acid output of approximately 28 g/L.
2. Conversion rates of 50% or higher can be realized with yields of over 30 %.

3. Corn stover and pig manure fermentation produces a large quantity of gas, especially at initiation, and an addition of 120 μL of 20 mL iodoform/L ethanol solution every other day is sufficient in preventing the production of methane.
4. The gas must be vented and measured every day to prevent an explosion.
5. Sonication produced no apparent effects in carboxylic acid concentration, athough there was an increase in the yield (g total acid/g VS fed).

RECCOMENDATIONS

1. I recommend that additional batch studies be conducted to test the dependency of the corn stover/pig manure system on nutrient addition. Nutrients are not preferable economically, and information on the loss of production incurred by omitting the nutrient medium would be useful.
2. Also, when beginning a fermentation train with corn stover and pig manure, the system is very volatile. The gas must be vented once, even twice a day for the first two weeks to prevent the fermentor from bursting.
3. Further sonication research is recommended. The production of batch tests may give more concrete initial results since there are fewer factors affecting a batch than continuous system.

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APPENDIX A

LIME TREATEMENT PROCEDURE

To perform a lime pretreatment, the following supplies are necessary:

- Biomass to be treated
- Calcium hydroxide (slaked lime)
- Carbon dioxide (CO_2)
- Distilled water
- Large stainless steel pan (approximately 24 × 16 × 4 inches)
- Metal stir
- One liter graduated cylinder
- pH paper

Step 1 Weigh and record the amount of the biomass to be treated into a large stainless steel pan.

Step 2 Weigh 0.1 grams of calcium hydroxide (slaked lime) per gram of biomass into the pan.

Step 3 Add 10 mL of water per gram of biomass to form a slurry, and mix well.

Step 4 Heat the slurry, allowing it to boil, for at least one hour. The time depends on the biomass type. Stir the mixture occasionally to assure thorough mixing, and do not let the slurry go dry. Add more water if necessary.

Step 5 Remove the pan from the heating apparatus and allow the slurry to cool to room temperature.

Step 6 Add more water if necessary to create a relatively liquid mixture.

Step 7 Bubble CO_2 through the mixture to neutralize the lime. Continue until the pH becomes approximately seven, as measured by pH paper. This process may take several hours. If foaming becomes a problem during this time, add seven drops of Dow Corning antifoam solution.

Step 8 Dry the neutralized mixture in an oven at 105 °C for two days to remove excess water.

APPENDIX B

FERMENTOR CONSTRUCTION

Fermentor assembly requires the use of several machine tools that may not be readily available. For this project, tools from several locations at Texas A&M University (College Station, Texas) were used. These tools are referenced throughout the proceeding steps, and their location is described.

To assemble one fermentor, the following supplies are necessary:

- 1-L centrifuge bottle
- Rubber stopper (Size 11)—must fit centrifuge bottle
- stainless steel Tubing (0.25-in. welded 304)
- Glass test tube (approximately ¾-inch outer diameter)
- Rubber septum and closure for test tube
- Rubber tubing (approximately ¼-inch outer diameter)
- Four ring clamp closures
- Rubber rings (approximately ½-inch outer diameter)
- Tube grease for lubrication

CUTTING AND BENDING TUBING

The metal tubing must be cut and bent to the specified lengths and angles using cutting and bending tools (Cater Mattil Laboratory, Texas A&M University, College Station, TX).

Step 1 Cut a 10-inch length of tubing and a 12-inch length of stainless steel tubing.

Step 2 Mark the 10-inch tubing at 4 inches and at 5 ½ inches with a marker.

Step 3 Mark the 12-inch tubing at 3 inches and at 6 inches.

Step 4 Bend the tube to the appropriate angles, as depicted by Figure B-1.

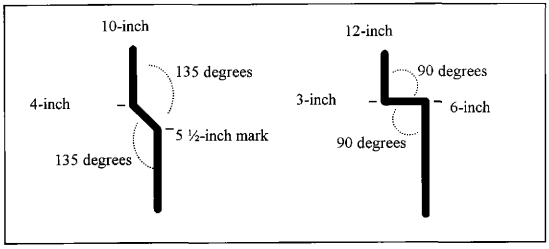

Figure B-1: Tube angles.

The two pieces should fit together as depicted in Figure B-2, and lie flat after the bending.

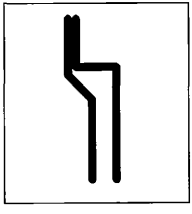

Figure B-2: Tube fitting.

DRILLING STOPPERS

To insert the stainless steel tubing and the glass tube for gas venting, three holes are placed in the rubber stopper. The drilling is done on a special stopper drill (Heep Center for Soil and Crop Sciences, Texas A&M University, College Station, TX).

Step 1 Drill one hole, ¾ inch in diameter, completely through the center of the stopper (*Figure B-3*).

Step 2 Drill two holes, ¼ inch in diameter, completely through the stopper on either side, and in line with the center hole (Figure B-3).

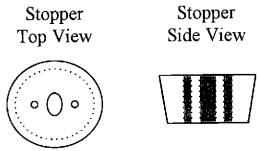

Figure B-3: Hole placement.

CUTTING THE GLASS TUBES

The glass tubes must be cut at approximately three inches and brought in to a glass shop to flare the bottom (Heep Center for Soil and Crop Sciences, Texas A&M University, College Station, TX). An example is depicted in Figure B-4.

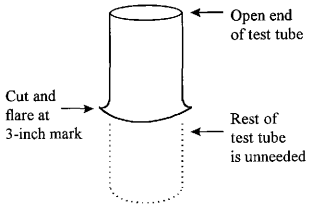

Figure B-4: Glass tube shaping.

ASSEMBLING THE FERMENTOR

Figure B-5 depicts a fully assembled fermentor for reference during assembly.

Step 1 Weld the ends of the stainless steel tube shut so no gas can exit through the end.

Step 2
- **A.** Lubricate the glass tube with grease.
- **B.** Combine the glass tube and stopper by inserting the non-flared end of the test tube up through the bottom of the stopper using a twisting motion.

Step 3
- **A.** Insert the rubber septum into the non-flared end of the glass tube.
- **B.** Secure with a metal closure

Step 4
- **A.** Place the stainless steel pieces in the configuration depicted in Figure B-2.
- **B.** Insert the stainless steel tubes into the stopper by bringing the bottom of the tube up through the bottom of the stopper.
- **C.** Secure the tubes with ring clamp closures.

Step 5 Bend the tubes that protrude above the stopper to 90 degree angles facing each other. Make sure to keep the original tube configuration below the stopper. NOTE: The tubes must be inserted into the stopper BEFORE making the final bends.

Step 6 Bind the pipes that protrude below the stopper with a rubber ring to keep them together.

Step 7 Cut a circle (approximately 1 ½ inches in diameter) from the lid of the 1-L bottle using a sharp blade.

Step 8 Insert the stopper apparatus into the 1-L bottle and secure with the bottle lid.

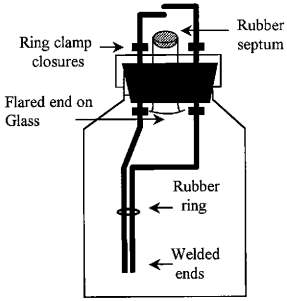

Figure B-5: Assembled fermentor.

APPENDIX C

NURIENTS AND MEDIA PREPARATION

MEDIA

The media of deoxygenated, distilled water contained cysteine hydrochloride and sodium sulfide. It was prepared by boiling distilled water under a nitrogen purge for five minutes, cooling, and adding the ingredients depicted in Table C-1 (Domke, 1999).

Table C-1: Media Preparation.

Additions	Amount (g/L distilled water)
Cysteine hydrochloride	0.275
Sodium sulfide	0.275

NUTRIENTS

The modified Caldwell and Bryant medium recommended by Ross (1988) is a liquid medium prepared by adding 1.4 g of the dried nutrient mixture listed in Table C-2 to 1 L of water.

Table C-2: Nutrients Preparation (Ross, 1998).

Component	Amount (g/100 g mixture)
K_2HPO_4	16.3
KH_2PO_4	16.3
$(NH_4)SO_4$	16.3
NaCl	32.6
$MgSO_4 \cdot H_2O$	6.8
$CaCl_2 \cdot H_2O$	4.4
HEPES (N-2-Hydroxyethyl piperazine-N`-2 ethanesulfonate)	0.86
Hemin	0.71
Nicotinamide	0.71
p-Aminobenzoic acid	0.71
Ca-pantothenate	0.71
Folic acid	0.35
Pyridoxal	0.35
Riboflavin	0.35
Thamin	0.35
Cyanocobalamin	0.14
Biotin	0.14
EDTA	0.35
$FeSO_4 \cdot 7H_2O$	0.14
$MnCl_2$	0.14
H_3BO_3	0.021
$CoCl_2$	0.014
$ZnSO_4 \cdot 7H_2O$	0.007
$NaMoO_4 \cdot 2H_2O$	0.0021
$NiCl_2$	0.0014
$CuCl_S$	0.0007

APPENDIX D

COUNTERCURRENT DOUBLE-CENTRIFUGE PROCEDURE

Each fermentation train conducted in this project ran in a countercurrent fashion accomplished through the double-centrifuge procedure developed by Ross (1998), Domke (1999) and others. A nitrogen purge should be utilized at all times when the fermentors are open to the atmosphere.

To run a countercurrent fermentation, the following supplies are necessary:

- Fermentation train (four fermentors: F1, F2, F3 and F4)
- Plastic weighing trays
- One pre-weighed portion of your biomass in the appropriate ratio
- Four pre-weighed portions of calcium carbonate
- Four pre-weighed portions of nutrients
- Four pre-weighed portions of urea
- Iodoform
- Deoxygenated water media
- Metal stir
- Four normal 1-L bottle lids
- Plastic test tube for sample
- Waste bottle for liquid and solids
- Graduated cylinder (200 mL)
- 100-mL beaker
- Nitrogen Purge

Step 1
A. Remove the fermentors from the incubator and allow them to cool.
B. Measure the gas production.

Step 2
A. Remove the lid from F1, and clean the excess biomass from the underside of the stopper.
B. Close the fermentor bottle with a regular 1-L bottle lid.
C. Repeat for Fermentors 2, 3, and 4.

Step 3
Centrifuge the fermentors for 15 minutes at 3500 rpm.

Step 4
A. Open F1 and pour the liquid into a graduated cylinder.
B. Record the weight and volume of the liquid.
C. Take a sample of the liquid and place it in a plastic test tube.

D. Place the remaining liquid in a labeled "liquid waste" bottle for the specific fermentation train.
E. Store the test tube and the bottle in the freezer for eventual analysis.

Step 5
A. Open F2, pour the liquid into F1, and stir.
B. Open F3, pour the liquid into F2, and stir.
C. Open F4, pour the liquid into F3, and stir.
D. Add 100 mL of deoxygenated water media (Appendix C) to F4.
E. Recap all fermentors.

Step 6 Centrifuge the fermentors for 15 minutes at 3500 rpm.

Step 7
A. Open F1 and pour the liquid into a clean 250-mL beaker.
B. Weigh F1, and record the weight.
C. Subtract the mass of fresh biomass from the desired mass of the fermentor. Subtract this value from the recorded mass of F1. This is the amount of solids that must be removed from F1.
D. Remove the appropriate amount of solids from F1.
E. Add the fresh biomass to F1.
F. Add one pre-weighed portion of calcium carbonate, nutrients and urea, and the necessary amount of iodoform to F1.
G. Pour the liquid from F1 (in the 250-mL beaker) back in to F1.
H. Open F2, pour the liquid into F1, and stir.
I. Replace the stopper on F1.

Step 9
A. Weigh F2, and record the mass.
B. Subtract the mass of solids removed from F1 from the desired mass of the fermentor. Subtract this number from the mass of F2. This is the amount of solids to be removed from F2.
C. Remove the appropriate mass of solids from F2.
D. Add the solids removed from F1 to F2.
E. Add one pre-weighed portion of calcium carbonate, nutrients and urea, and the necessary amount of iodoform to F2.
F. Open F3 and pour the liquid into F2.
G. Stir F2 thoroughly.
H. Replace the lid apparatus (from the holding tray) on F2.

Step 10 Repeat Step 9 for F3, subtracting the weight of solids removed from F2 in 9-B, adding the solids removed from F2 in 9-E, and adding the liquid from F4 in 9-H.

Step 11
A. Repeat steps 9-A through 9-G for F4, subtracting the weight of solids removed from F3 in 9-B, adding the solids removed from F3 in 9-E.

B. Add 100 mL of deoxygenated water media to F4.
C. Stir F4 thoroughly.
D. Replace the lid apparatus (from the holding tray) on F4.

Step 12

A. Place the solids removed from F4 in a labeled waste bottle for solids.
B. Store the bottle in the freezer for eventual analysis.
C. Replace the fermentors in the fermentor oven.

APPENDIX E

VOLATILE SOLIDS ANALYSIS TECHNIQUES

LIQUID ANALYSIS

The liquid analysis is performed on the liquid from fermentor one in a technique developed by Ross (1998).

To perform a liquid analysis, the following supplies are necessary:

- Full liquid collection bottle
- Empty 1-L centrifuge bottle
- Two 150 mL or larger crucibles
- Calcium hydroxide
- Metal stir

Step 1 Record the weight (W1) of the full liquids collection bottle with no lid.

Step 2 Cap the bottle and centrifuge it for 10 minutes at 3500 rpm.

Step 3 Record the weight (W3) of an empty 1-L centrifuge bottle (B2).

Step 4 Add approximately 3 grams of calcium hydroxide (slaked lime) to B2 and record the weight (W4).

Step 5 Add approximately 100 g of the liquid from the centrifuged liquids collection bottle and record the weight (W5).

Step 6 Record the label and weight (W6) of a crucible (C1).

Step 7 Add a sample of the slurry prepared in Step 4 to C1 and record the weight (W7).

Step 8 Place C1 in a drying oven at 105 °C for at least 48 hours. Record the dry weight (W8) of C1.

Step 9 Place C2 in an ashing oven at 550 °C for at least 3 hours. Record the ash weight (W9) of the C2.

Step 10 Empty the rest of the liquid in the liquids collection bottle into the sink carefully, so as not to lose any of the solids at the bottom of the bottle. It is acceptable to leave a small amount of liquid to prevent solid loss. Record the weight (W10) of the bottle and the solids.

Step 11 Record the label and weight (W11) of a crucible (C2).

Step 12 Add approximately 3 grams of calcium hydroxide (slaked lime) to C2 and record the weight (W12).

Step 13 Add approximately 75 g of the solids from liquids collection bottle to C2, mix thoroughly and record the weight (W13).

Step 14 Place C2 in a drying oven at 105 °C for at least 48 hours. Record the dry weight of C2 (W14).

Step 15 Place C2 in an ashing oven at 550 °C for at least 3 hours. Record the ash weight of the C2 (W5).

Using the values of W1 to W15 recorded in the proceeding instructions, the VS dissolved in the liquid can be calculated as follows:

$$VS_{dissolved} = \frac{(W8 - W9)}{\left(\frac{W7 - W6}{W5 - W3}\right) \times \left(\frac{W5 - W4}{W1 - W10}\right)}$$

Additionally, the particulate VS present in the liquid is calculated by:

$$VS_{particulate} = \frac{(W14 - W15)}{\left(\frac{W13 - W12}{W10 - W16}\right)}$$

Therefore, the total amount of VS present in the liquid is:

$$VS_{total} = VS_{dissolved} + VS_{particulate}$$

SOLID ANALYSIS

The solid analysis is performed on the residue from fermentor four in a technique developed by Ross (1998).

To perform a liquid analysis, the following supplies are necessary:

- Full solids collection bottle
- 150-mL or larger crucible
- Metal stir
- Small metal pan

Step 1 Record the weight (W1) of a full solids collection bottle with no lid.

Step 2 Empty solids into the small pan and mix well.

Step 3 Record the label and weight (W3) of a crucible.

Step 4 Place a representative sample (~100 g) of the solids into the crucible and record the weight (W4).

Step 5 Place the crucible in a drying oven at 105 °C for at least 48 hours. Record the dry weight of the crucible (W5).

Step 6 Place the crucible in an ashing oven at 550 °C for at least three hours. Record the ash weight of the crucible (W6).

Step 7 Record the weight (W7) of the empty solids collection bottle.

The VS in the solid residue is calculated as follows:

$$VS_{residue} = \frac{(W5 - W6)}{\left(\frac{W4 - W3}{W1 - W7}\right)}$$

VITA

Amanda Black was born in Houston, TX and moved to College Station in August of 1996 to attend Texas A&M University where she was the recipient of a Presidents Endowed Scholarship. She has worked in the laboratory of Dr. Mark Holtzapple since the summer of 1998 and will continue to do so until she graduates in May 2001 with a Bachelor of Science in Chemical Engineering. Her permanent address is 2951 Marina Bay Dr. Suite 130-119, League City, TX 77573.